PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>W 3936-007 | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/SE 03/01211 | International filing date *(day/month/year)*<br>11.07.2003 | Priority date *(day/month/year)*<br>26.07.2002 |
| International Patent Classification (IPC) or both national classification and IPC<br>C12Q1/37 | | |
| Applicant<br>WIESLAB AB et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 12.02.2004 | 27.10.2004 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized Officer<br>Herrero, M<br>Telephone No. +49 89 2399-8542 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/SE 03/01211

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

1-32 as published

**Claims, Numbers**

1-24 received on 08.10.2004 with letter of 04.10.2004

**Drawings, Sheets**

1/11-11/11 as published

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

   **see separate sheet**

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-24 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-24 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1-24 |
| | No: | Claims | |

2. Citations and explanations

   **see separate sheet**

## SECTION I

6. Additional observations:

The amended Claims 1-24 filed with the letter dated 04.10.04 have their basis in the originally filed application, and therefore do not contravene Art. 34(2)(b) PCT.

## SECTION V

2. CITATIONS AND EXPLANATIONS

2.1 In view of the first priority document pertaining to the present application, the scientific publication J. Immunol. Methods **268**:149-157 (publication date 15.10.02), which should have been classified in the search report under the P category, is not to be regarded as state of the art according to Rule 64 (1) PCT as the date of priority of 26.07.02 is validly claimed for the relevant parts of the application.

2.2 Novelty and inventive step Arts. 33(2) and (3) PCT

Taking into account the documents cited in the International Search Report the subject-matter encompassed by the newly filed Claims 1-8 (methods) and 9-24 (kits) is considered to be novel, as required by Art. 33(2) PCT.

Moreover, having regard to the arguments presented in the Applicants' letter dated 04.10.04, it is agreed that the methods and kits encompassed by present reformulated Claims 1-24 may be regarded as inventive (Art. 33(3) PCT).

The problem to be solved by the present application relates to the provision of a method suitable for the functional identification at physiological conditions of deficiencies in the lectin pathway of the complement system of a mammal, including humans (cf page 7, lines 23-28 of the description).

To achieve this purpose, the pursued method should allow for a specific assessment of the complete lectin pathway of complement activation until formation of the C5b-C9 complex, i.e. possible false positive results resulting from

the activation of the classical pathway (CP) and/or the alternative pathway (AP) should be eliminated.

In the working approach to solve the underlying technical problem hereby proposed according to Claims 1-8, the activation of the two non-assayed pathways CP and AP is effectively prevented in the sample under study employing the procedural steps (a) and (b), respectively. Subsequently, the activation of the lectin pathway at physiological conditions is determined in the presence of an antibody against autologous C5b-C9 complex according to the procedural steps (d) and (e).

A procedure suitable for functionally determining at physiological conditions deficiencies in the lectin pathway of the complement system, in which a sample of mammalian blood, serum, plasma or another body fluid of a mammal is assayed by means of performing the working steps (a) to (e) which characterize the hereby claimed methods (see also supporting description on page 8, lines 28-34 bridging over pages 9-15 and page 16, lines 1-7), is considered to be non-obviously derivable from the available prior art.

Likewise, the kits according to Claims 9-24, which comprise all the relevant components required to carry out the procedures defined in Claims 1-8, are considered to satisfy the inventive step criterion set forth by Art. 33(3) PCT.

2.3 Further comments

(i) Present Claims 4 and 13 refer to an anti-C1q monoclonal antibody identified by the arbitrary denomination 2204, which seems to be technically meaningless to the person skilled in the art. Thus Claims 4 and 13 (and any claim dependent thereon) do not comply with the clarity requirements of Art. 6 PCT.

Moreover, in the absence of evidence substantiating the public availability of the anti-C1q monoclonal antibody hereby denominated 2204, it is at present not possible to ascertain whether the subject-matter of Claims 4 and 13 (and of any claim dependent thereof) fulfills the requirements of sufficiency of disclosure imposed by Art. 5 PCT.

(ii) In view of the supporting description e.g. on page 20, lines 1-12, Claim 29 as originally filed and present amended Claim 16, it is apparent that Claims 7 and 8 presently on file have been wrongly formulated (Art. 6 PCT). Thus, it appears that present Claims 7 and 8 should have referred, for instance, to:

"The method according to claim 6, wherein the step in (d) comprises adding a second antibody against the first antibody, wherein said second antibody is a labeled antibody" (cf Claim 7).

"The method according to claim 6 wherein the first antibody is a labeled antibody" (cf Claim 8)

(iii) In line with the foregoing discussion it seems that Claim 17 should have referred back to claims 9-15 instead of to claims 9-16.

(iv) With respect to Claims 4 and 5 it is noted that the use of expressions like "such as" (or "for example" or "preferably"...) has no limiting effect on the scope of said claim, i.e. the feature(s) following such expressions is(are) to be regarded as entirely optional (cf PCT Guidelines, C-III, 4.6).

(v) The use of the vague term "molecules" in the definition of component (b) of the intended kit according to independent Claim 9 renders the scope of the claim unclear, contrary to Art. 6 PCT.

(vi) For the sake of clarity (Art. 6 PCT) the actual meaning of the acronym MBL, i.e. mannan-binding lectin (see page 15, line 17) should have been given at its first appearance in the claims (i.e. in Claim 1).

(vii) It appears that the third line of Claim 5 was meant to read "fucose" instead of "fructose" (cf page 16, line 21). See also that Claim 9, line 7 reads "... an first antibody..." and Claim 18, line 2 reads "fluoroscent".

PATENT CLAIMS

1. An *in vitro* method of functionally determining at physiological conditions deficiencies in the lectin pathway of the complement system, employing a sample of mammalian blood, serum,plasma, or another body fluid obtained from a mammal, the method comprising the steps of

(a) adding an C1 complex inhibitor selected from the group consisting of proteins, peptides or immunoglobulins against C1q, C1r or C1s;

(b) diluting the sample to inhibit the activation of the alternative pathway;

(c) adding a MBL or ficolin binding carbohydrate activating the lectin pathway in the sample;

(d) adding an first antibody against the autologous C5b-9 complex and

(e) determining the activation of the lectin pathway at the physiological condition by measuring the autologous C5b-9 complex.

2. The method according to claim 1, wherein the inhibitor in step (a) is selected from the group consisting of C1 inhibitor, CRT, C1Qr, E.coli C1g binding protein, gC1qR, ghB3, decorin, chondroitin sulphate proteoglycan, surfactant protein A and HNP-1.

3. The method according to claim 1, wherein the inhibitor in step (a) is selected from the group consisting of TDGDKAFVDFLSDEIKEE, KDIRCKDD, AEAKAKA, VQVHNAKTKPR, WY, CEGPFGPRHDLTFCW and LEQGENVFLQATLL.

4. The method according to claim 1, wherein the inhibitor in step (a) is selected from the group consisting of polyclonal and monoclonal antibodies, such as IVIg and anti-C1q mAB 2204.

5. The method according to any of preceding claims, wherein the carbohydrate in step (c) is selected from the group consisting of mannose, fructose, mannan such as glucomannan and galactomannan, synthetic carbohydrate and microbial polysaccharide.

6. The method according to any of preceding claims, wherein the first antibody in step (d) is a polyclonal or a monoclonal antibody.

7. The method according to claim 6, wherein the step in (d) comprises adding a second antibody against the first antibody.

8. The method according to any of preceding claims, wherein the first or the second antibody is a labeled antibody.

9. A kit for functionally determining in a body fluid from a mammal deficiencies in the lectin pathway of the complement system, which kit comprises (a) an inert carrier and a MBL or ficolin binding carbohydrate (b) a diluent comprising a C1 complex inhibitor selected from the group consisting of molecules, peptides, proteases or immunoglobulins against C1q, C1r or C1s and (c) an first antibody against the autologous C5b-9 complex.

10. The kit according to claim 9, wherein the carbohydrate in (a) is selected from the group consisting of mannose, fructose, mannan such as glucomannan and galactormannan, synthetic carbohydrate and microbial polysaccharide.

11. The kit according to claim 9-10, wherein the inhibitor in (b) is selected from the group consisting of C1 inhibitor, CRT, C1Qr, E.coli C1g binding protein, gC1qR, ghB3, decorin, chondroitin sulphate proteoglycan, surfactant protein A and HNP-1.

12. The kit according to claim 9-11, wherein the inhibitor in (b) is selected from the group consisting of the peptides, TDGDKAFVDFLSDEIKEE, KDIRCKDD, AEAKAKA, VQVHNAKTKPR,WY,CEGPFGPRHDLTFCW and LEQGENVFLQATLL.

13. The kit according to claim 9-12, wherein the inhibitor in (b) is selected from the group consisting of polyclonal and monoclonal antibodies, such as IVIg and anti-C1q mAB 2204.

14. The kit according to any of claims 9-13, wherein the first antibody in (c) is a polyclonal or monoclonal antibody.

15. The kit according to any of claims 9-14, wherein the carbohydrate in (a) is coated on the inert carrier.

16. The kit according to any of claims 9-15, wherein the first antibody in (c) is a labeled antibody.

17. The kit according to any of claims 9-16, wherein the kit further comprises a labeled second antibody (d) against the antibody in (c).

18. The kit according to claim 17, wherein the label is a fluoroscent or an enzyme label.

19. The kit according to claim 9-18, wherein the kit further comprises an enzyme substrate (e).

20. The kit according to any of claims 9-19, wherein the kit further comprises a washing solution (f).

21. The kit according to any of claims 9-20, wherein the kit further comprises a normal body liquid from a mammal (g).

22. The kit according to claim 21, wherein the normal body liquid (g) is a human serum.

23. The kit according to any of claims 10-23, wherein the kit further comprises an inactivated normal body liquid from a mammal (h).

24. The kit according to claim 24, wherein the inactivated normal body liquid (h) is heat inactivated human serum.